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(54) Title: METHOD AND COMPOSITION FOR THE REGULATION OF HEPATIC AND EXTRAHEPATIC PRODUCTION OF INSULIN-LIKE GROWTH FACTOR-1

(57) Abstract

Method for separately regulating hepatic and extrahepatic production and insulin-like growth factor-1 (IGF-1). The levels of IGF-1 in sera can be significantly reduced without reducingt the desired actions of IGF-1. This is achieved by separately regulating the systemic and local distribution of IGF-1 without affecting body growth. The invention also relates to the treatment of obesity. It further refers to the treatment of retinopathy in diabetic patients. Inhibition of the biological effects exerted by IGF-I in blood circulation could be obtained by injection of IGF-I antagonists, IGF binding proteins, or thyrohostins into blood circulation or by blockade of hepatic IGF-I information, for instance by oral treatment with estrogenic agents or by local treatment with small nucleotide molecules.

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Method and composition for the regulation of hepatic and extrahepatic production of insulin-like growth factor-1

DESCRIPTION

Technical field

The present invention relates to a method for separately regulating hepatic and extrahepatic production of insulin-like growth factor-1 (IGF-1). It also relates to the treatment of obesity; and use of inhibitors of hepatic and circulating IGF-1 for the production of pharmaceuticals for preventing or reducing obesity. The invention further refers to the treatment of retinopathy in diabetic patients and use of inhibitors of hepatic and circulating IGF-1 for the production of pharmaceuticals for preventing or reducing retinopathy.

Background of the invention

Insulin-like growth factor-1 (IGF-1)

Insulin-like growth factor-1 (IGF-1) is a growth factor that can stimulate to cell division. Chemically it is similar to insulin and it has weak antidiabetic effects including suppression of blood glucose. It exerts its biological effects through the ligand-specific IGF-1 receptor, which belongs to the tyrosine kinase family of cell membrane receptors (Froesh et al., 1985, Jones & Clemmons, 1995, Stewart & Rotwein, 1996).

IGF-1 is present in large quantities in blood circulation. The results obtained by us (Sjögren et al., 1999) as well as others (Froesh et al. 1985) demonstrate that the majority of all circulating IGF-1 is liver derived. The circulating IGF-1 is to a large part bound in a ternary complex to two other proteins, acid labile subunit (ALS) and IGF binding protein-3 (IGFBP-3), which also are produced by the liver (Jones & Clemmons, 1995, Stewart & Rothwein, 1996).

Growth hormone (GH)

Growth hormone (GH), a major stimulator of body growth released from the pituitary, stimulates the production of IGF-1 in the liver but also locally in peripheral target tissues of the growth promoting effect of GH (Isaksson et al., 1982, D'Ercole et al., 1984, Froesh et al., 1985, Daughaday & Rotwein, 1989). In almost all species, GH is secreted in a more or less

pulsatile fashion (Jansson et al., 1985). The degree of pulsatility seems to be affected by several factors such as age and gender (Jansson et al., 1985, Jaffe et al., 1998). There are indications that a more pulsatile GH secretory pattern is more efficient than a continuous pattern in promoting body growth (Jansson et al., 1982, Clark et al., 1985, Jansson et al., 1985). Moreover, a pulsatile GH pattern seems to be more efficient for initiation of IGF-1 expression in cartilage and skeletal muscle, i.e., extrahepatic target tissues for the growth promoting effects of GH (Isgaard et al., 1988).

In contrast, continuous GH promotes several effects in the liver (Jansson et al., 1985, Gustafsson et al., 1983). For instance, a continuous GH pattern may be more effective than a pulsatile GH secretion in inducing liver growth as well as hepatic IGF-1 production. The latter effect could in turn result in higher levels of liver derived IGF-1 in blood circulation after continuous GH treatment (Clark et al., 1995, Orlowski & Chernausek, 1988). The physiological role of liver derived, circulating IGF-1 versus locally produced IGF-1 has been unclear.

In addition to GH, estrogens may regulate liver production of IGF-1. Orally administered estrogens which have been reported to inhibit the hepatic IGF-1 production via a so called first passage effect exerted directly on the liver (O'Sullivan et al., 1998). On the other hand food intake is a stimulator of hepatic IGF-1 production (Stewart & Rothwein, 1996).

IGF-1 and postnatal body growth

IGF-1 and IGF-2 are assumed to be of crucial importance for body growth. Experiments in which the IGF-1 and IGF-2 genes have been deleted in mice indicate that both IGF-1 and IGF-2 are of importance for growth before birth (Powel-Braxton et al., 1993, Liu et al., 1993, Baker et al., 1993). These gene knock out experiments as well as studies in which IGF-2 has been expressed in very high levels in transgenic mice indicate that IGF-2 is not important for postnatal growth. Moreover, treatment with IGF-2 does not reverse the growth inhibition in GH deficient rats indicating that IGF-2 is not of major importance for the growth stimulating effect of endogenous GH during adult life (see Stewart & Rothwein, 1996).

Experiments with IGF-1 gene deletion in mice also showed that IGF-1, unlike IGF-2, is of importance for growth after birth (Powel-Braxton et al., 1993, Liu et al., 1993, Baker et al. 1993). A patient with a deletion of the IGF-I gene demonstrated postnatal growth failure in

addition to intrauterine growth retardation (Woods et al., 1996). Moreover, IGF-1 can partly, but not completely, substitute for GH in stimulation of body growth (Daughaday & Rotwein, 1989, Stewart & Rothwein, 1996). During the last 10 years it has become evident that systemic administration of IGF-I to GH-deficient/ GH receptor-mutated animals and man, stimulates body growth. These findings suggest that both IGF-I and GH have the capacity to stimulate body growth *in vivo* (Guler et al., 1988, Laron et al., 1992, Walker et al., 1991).

Liver derived, circulating IGF-1 versus locally produced IGF-1 in regulation of body growth

It has recently been shown that local treatment with IGF-1 can reverse age-related loss of skeletal muscle without changing circulating IGF-1 levels (Barton-Davis et al., 1998). It is however questionable if this finding applies to the growth of other extrahepatic organs.

The findings that local administration of GH or IGF-1 into the growth plate of long bones could induce bone growth in rats without endogenous GH suggested that GH also could exert growth-promoting effects that are liver independent (Isaksson et al. 1982). Based on this finding, it has been stated later that GH can exert a local growth promoting effect (Ohlsson et al., 1998). However, these finding only showed comparatively small effects of GH given pharmacologically to animals that were hypersensitive to GH due to long term lack of endogenous GH. Therefore, it has been supposed that local production of IGF-1 could contribute to only 20 % (Daughaday & Rothwein 1989) or at most 50-60 % (Isaksson et al., 1987) of total GH stimulated body growth. In line with this assumption, it has been claimed that therapies that enhance the levels of circulating IGF-1 also reverses catabolic conditions such as bone loss associated with ageing (US Pat 5240961).

Suppression of hepatic IGF-1 production and circulating IGF-1

Several different methods to inhibit IGF-1 production in the liver have been described. These methods include oral estrogen treatment (O'Sullivan et al., 1998) and antisense oligonucleotides directed against IGF-1 mRNA in the liver. Several methods to selectively affect gene expression in general in the liver have been described. These methods for so-called gene therapy include administration of substances selectively to liver cells by use of viruses such as adenovirus and retrovirus (Ferry et al., 1998). It may also be possible to neutralise circulating IGF-1 by treatment with IGF-1 antagonists and IGF-1 blocking antibodies. IGF binding proteins, a group of endogenous factors could also be used

pharmacologically to inhibit deleterious effects of IGF, as previously claimed (US Pat 5681818, US Pat 5693754, US Pat 5840673). Moreover, IGF-1 receptor specific tyrphostins could inhibit the effects of IGF-1 (Párrizas et al., 1997).

IGF-1 and tumours

The results of epidemiological studies suggest that the levels of circulating IGF-1 may play a role for tumour development, for instance for breast and prostatic tumours (Chan et al., 1998, Hankinson et al., 1998).

As mentioned above, IGF does not affect the proliferation of hepatocytes, since these cells have no IGF-1 receptors. However, some tumours cells originating from hepatocytes may get more IGF-1 receptors, a feature that could provide them with a growth advantage (Caro et al., 1988). In vivo, overexpression of IGF-2, which also act via IGF-1 receptors, has been found in pre-neoplastic noduli and hepatic tumours (Stewart & Rothwein 1996). Lung cancer cells that have higher number of IGF-1 receptors and are more responsive to IGF are also more prone to get implanted as metastases in the liver (Long et al., 1994).

IGF-1 and liver cirrhosis

There are indications in the literature that IGF-1 may play a role also for the development of liver cirrhosis, a condition with accumulation of collagen and connective tissue in the liver. As mentioned above, there are very few IGF-1 receptors on normal, non-tumour hepatocytes (Froesh et al., 1985). Instead, the cirrhosis promoting effect of IGF-1 seems to be mediated by receptors on another cell type in the liver, i.e., the so-called stellate cells. During liver cirrhosis, these cells begin to proliferate and to produce collagen and other components of connective tissue that cause this illness. This proliferation and collagen production may partly be due to stimulation by IGF-1 (Pinzani et al., 1990).

IGF-1 and retinopathy

It has been shown that upregulation of serum IGF-1 precedes and probably also causes retinal deterioration in some diabetic patients. (Chantelau E., 1998)

Summary of the invention

It has hitherto not been held practically possible to separately regulate the systemic and local distribution of IGF-1 or, in other words, to obtain the beneficial effects of inhibition of circulating hepatic IGF-1 without affecting body growth. Also, the physiological role of liver derived, circulating IGF-1 versus locally produced IGF-1 has been unclear. The present inventors surprisingly found that the levels of IGF-1 in serum can be significantly reduced without reducing the desired actions of IGF-1. It has further according to the invention been found that it is possible to influence body composition via a delivery of a substance modulating the effect of circulating liver-derived IGF-I. Such beneficial effects on body composition include, but are not limited to, treatment of obesity. It may also be possible to treat or reduce retinopathy in diabetic patients. The present results also surprisingly indicate that it is possible to achieve normal body growth in the face of a 75–80 % reduction of circulating IFG-1.

Short description of the drawings

The invention will be illustrated in closer detail below, with reference to the attached drawings, in which

Fig. 1 shows body weight growth curves for male (A) and female (B) mice after inactivation of the IGF-1 gene, compared with controls, and

Fig. 2 shows the liver / body weight ratio for IGF-1 inactivated mice compared to controls.

Description of the invention

The suppression of liver IGF-1 production caused a 75-80% decrease of the levels of IGF-1 in blood circulation. There was no substantial effect on body growth, while liver growth was increased in relation to body weight.

Firstly, these data indicate that it is possible to obtain beneficial effects of suppression of hepatic IGF- production and the levels of circulating IGF-1 without getting undesirable side effects such as stunted body growth and catabolism. Such beneficial effects include, but are not limited to, stimulated liver growth and function. Inhibition of the biological effects exerted by IGF-1 in blood circulation could be obtained by injection of IGF-1 antagonists,

IGF binding proteins, e.g. IGF binding protein III, or thyraphostins into blood circulation or by blockade of hepatic IGF-1 formation, for instance by oral treatment with estrogenic agents or by local treatment with small nucleotide molecules.

Secondly, accordingly to an embodiment of the present invention, it is possible to obtain close to normal body growth without stimulating hepatic IGF-1 production. Such stimulation of body growth could be due to direct effects of growth hormone (GH) on extrahepatic tissues and may include stimulation of local IGF-1 in these extrahepatic tissues. One way to get selective effects of GH on IGF-1 production and growth of extrahepatic target tissues could be by administration of GH in infrequent injections rather than more continuously. Alternatively, IGF-1 could be linked to molecules, including IGF binding proteins, with a high affinity to extrahepatic tissues.

Old LI-IGF-I -/- mice are leaner than control mice. This result indicates that it is possible to influence body composition via a delivery of a substance modulating the effect of circulating liver-derived IGF-I. Such beneficial effects on body composition include, but are not limited to, treatment of obesity. Inhibition of the biological effects exerted by IGF-I in blood circulation could be obtained by injection of IGF-I antagonists, IGF binding proteins, or thyrohostins into blood circulation or by blockade of hepatic IGF-I formation, for instance by oral treatment with estrogenic agents or by local treatment with small nucleotide molecules.

Examples

The Cre/loxP recombination system, which has been shown to inactivate genes *in vivo* in a tissue-specific and inducible manner (Gu et al. 1994, Kuhn et al. 1995), was used to create mice with a selective and inducible knock out of the IGF-1 gene in the liver (LI-IGF-I -/- mice). These mice were generated by mating of two mouse strains. These strains were the MxCre31 which were provided by Ralph Kuhn and Claus Rajewsky at the University of Cologne and the IGF-1 loxP mice with exon 4 of the IGF-I gene flanked with loxP sites generated by Derek LeRoith and coworkers at NIH (Liu et al., 1998).

A liver-specific inactivation of IGF-I was induced in offspring homozygous for loxP and heterozygous for Mx-Cre by treatment with interferon (INF) at 24-28 days of age. The INF induces the enzyme Cre recombinase in these mice, since the Cre recombinase gene is under

the control of an INF-responsive promoter (Mx-Cre). This treatment has previously been shown to cause almost 100% recombination in the liver and a partial recombination in the spleen while the recombination in peripheral tissues like muscle, fat, kidney, heart and bone is low (Gu et al., 1994, Kuehn et al., 1995, Sjögren et al., 1999). Neither Cre-expression nor INF-treatment by themselves regulated any of the parameters described in the present study.

The efficiency of recombination was studied by Southern blot. The recombination in the liver was higher than 90 % and a complete recombination was found in purified hepatocytes in LI-IGF-I -/- mice. A less than 20 % recombination was found in all other tissues except the spleen where 65% recombination was found. No recombination was found in Cre mice not induced with INF. IGF-I mRNA levels were decreased more than 90% in liver while no significant effect was seen in other tissues except in the spleen where IGF-1 mRNA was decreased by about 60%. Furthermore, the content of immunoreactive IGF-I was decreased with more than 90% in purified hepatocytes from LI-IGF-I -/- mice, reaching the detection limit of the assay used. These data demonstrate that a specific inactivation of IGF-I expression was obtained in the liver (and to a lesser extent in the spleen, an organ not supposed to be of importance for postnatal growth).

Serum IGF-I levels decreased dramatically in mice with liver specific IGF-1 gene deletion. The effect was seen within one week after INF-induction and was still present 53 days later. Thus, liver-derived IGF-I is the main determinant of serum IGF-I levels. The present results showed that at least 75% of all IGF-I in mouse serum is derived from the liver. This finding is in line with the results of previous indirect calculations based on the IGF-I production rate in isolated rat liver (Froesh et al., 1985).

The decrease in serum IGF-I levels was followed by a concomitant increase in serum GH levels. These results show that circulating, liver-derived IGF-I exerts a negative feedback regulation of the pulsatile GH secretory pattern.

The effect of hepatic IGF-1 depletion on body growth was also investigated. Cre expression and subsequent IGF-1 gene inactivation was induced by administration of INF at 24-28 days of age. The body weight growth was monitored the following 53 days. Values are given as means $\pm$ SEM. No significant difference in weight gain was seen between LI-IGF-/- mice and control mice (Fig. 1). Similar results were seen in both male and female mice. There was

essentially no effect on the size of the heart (106% of control weight); the spleen (198% of control weight); two long bones femur (96% of control length); and tibia (99% of control length) at the time of sacrifice of the mice. Thus, the body growth was normal or almost normal in the LI-IGF-I-/- mice, demonstrating that liver-derived IGF-I is not required for postnatal growth.

Some visceral organs from mice with IGF-I inactivated from day 24 to 77 were weighed and related to total body weight. Values are expressed as percent of control and given as means ± SEM. The relative spleen and heart sizes were not significantly changed in LI-IGF-I-/- mice compared with control mice. Interestingly, the liver was larger in LI-IGF-I-/- mice compared with control mice (Fig. 2). Thus, the endocrine status in the LI-IGF-I-/- mice with very low serum IGF-I levels and incresed serum levels of GH in associated with normal total body growth but increased liver growth.

Unexpectedly it was found that between 100 and 300 days of age mice with liver specific IGF-1 gene deletion got less obese than control mice. Measurement of BMI, resulted in a BMI of 0,65 ± 0,02 g/ cm^2 for LI-IGF-I -/- mice compared to control mice which had a BMI of 0,73 ± 0,03 g/ cm^2, $p<0.05$.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.

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US Patent Publication No US 5,681,818 (Oct 28, 1997) Therapeutic uses of human somatomedin carrier proteins.

US Patent Publication No US 5,693,754 (Dec 2, 1997): Inhibitory binding protein for insulin-like growth factors.

US Patent Publication No US 5,840,673 (Nov 24, 1998): Insulin-like growth factor binding protein 3 (IGF-BP3) in treatment of p53-related tumours.

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CLAIMS

1. Method for the regulation of the systemic/ local distribution ratio of IGF-1 in mammals,
characterized in,
that at least one inhibitor of hepatic IGF-1 production is administered in a therapeutically effective dose to achieve the desired effect without an inhibitory effect on body growth and/or anabolism.

2. Method for inhibiting the development of liver cirrhosis,
characterized in,
that at least one inhibitor of hepatic IGF-1 production is administered in a therapeutically effective dose to achieve the desired effect without an inhibitory effect on body growth and/or anabolism.

3. Method for inhibiting the development of IGF dependent tumours,
characterized in,
that at least one inhibitor of hepatic IGF-1 production is administered in a therapeutically effective dose to achieve the desired effect without an inhibitory effect on body growth and/or anabolism.

4. Method for treating obesity by administering a therapeutically effective amount of a hepatic IGF-I inhibiting agent.

5. Method for treating retinopathy in diabetic patients by administering a therapeutically effective amount of a hepatic IGF-I inhibiting agent.

6. Method according to any of the claims 1-5, wherein the inhibiting agent is an inhibitor of hepatic IGF-I production.

7. Method according to any of the claims 1-5, wherein the inhibiting agent is a high or low affinity IGF binding protein.

8. Method according to any of the claims 1-5, wherein the inhibiting agent is an IGF-I receptor antagonist.

9. Method for any of the purposes described in the claims 1-5, **characterized** in, that agonists binding to and activating the same receptor proteins as estradiol are administered in a therapeutically effective dose to achieve the desired effect without an inhibitory effect on body growth and/or anabolism.

10. Method for any of the purposes described in the claims 1-5, **characterized** in, that small nucleic acid molecules for inhibiting IGF-1 gene expression are administered to a patient.

11. Method for any of the purposes described in the claims 1-5, **characterized** in, that said method includes the use of liver specific vectors including liver specific viruses to target substances to the liver specific vectors including liver specific viruses to target substances to the liver to obtain inhibition of IGF-1 production specifically in the liver.

12. Use of inhibitors of hepatic IGF-1 production for the manufacture of pharmaceuticals for the regulation of the systemic / local distribution ratio of IGF-1 in mammals.

13. Use of inhibitors of hepatic IGF-1 production for the manufacture of pharmaceuticals for enhancing weight and function of the intact liver with no or little concomitant inhibitory effect on body growth in mammals.

14. Use of inhibitors of hepatic IGF-1 production for the manufacture of pharmaceuticals for enhancing weight of the intact liver with no or little concomitant inhibitory effect on body growth in mammals.

15. Use of inhibitors of hepatic IGF-1 production for the manufacture of pharmaceuticals for inhibiting the development of liver cirrhosis with no or little concomitant inhibitory effect on body growth in mammals.

16. Use of inhibitors of hepatic IGF-1 production for the manufacture of pharmaceuticals for inhibiting the development of IGF dependent tumours with no or little concomitant inhibitory effect on body growth in mammals.

17. Use according to claim 16, specifically for IGF dependent tumours in the liver.

18. Use of agonists binding to and activating the same receptor proteins as estradiol for the manufacture of pharmaceuticals for inhibiting liver IGF-1 production and for any one of the following therapeutic purposes: enhancing weight and function of the intact liver; the inhibition of the development of IGF dependent tumours; inhibition of the development of liver cirrhosis; without an inhibitory effect on body growth in mammals.

19. Use of small nucleic acid molecules for the manufacture of pharmaceuticals for inhibiting IGF-1 gene expression and thereby IGF-1 production in the liver and for any one of the following therapeutic purposes; enhancing weight and function of the intact liver; the inhibition of the development of IGF dependent tumours; inhibition of the development of liver cirrhosis; without an inhibitory effect on body growth in mammals.

20. Use of high and low affinity IGF binding proteins, binding to IGF-1, for the manufacture of pharmaceuticals for any one of the purposes described in the claims 1 - 5.

21. Use if IGF binding protein III for the manufacture of pharmaceuticals for any one of the purposes described in the claims 1 –5.

22. Method to specifically stimulate growth in peripheral target organs of growth hormone to more than 20-50% of the maximal effect of GH on body growth with a concomitant small effect on hepatic IFG-1 production,
characterized in,
that at least one stimulator of peripheral extrahepatic IGF-1 gene expression is administered to a patient, optionally simultaneously with intermittent administration of GH.

23. Method to specifically stimulate growth in peripheral target organs of growth hormone to more than 20-50% of the maximal effect of GH on body growth with a concomitant small effect on the circulating levels of IGF-1,
characterized in,
that at least one stimulator of peripheral extrahepatic IGF-1 gene expression is administered to a patient, optionally simultaneously with intermittent administration of GH.

24. Method to specifically stimulate body growth with a concomitant small effect on hepatic IGF-1 production,
characterized in,
that at least one stimulator of peripheral extrahepatic IGF-1 gene expression is administered to a patient, optionally simultaneously with intermittent administration of GH.

25. Method to specifically stimulate body growth with a concomitant small effect on the circulating levels of IGF-1,
characterized in,
that at least one stimulator of peripheral extrahepatic IGF-1 gene expression is administered to a patient, optionally simultaneously with intermittent administration of GH.

26. Method to specifically stimulate anabolism in peripheral target organs of growth hormone with a concomitant small effect on hepatic IGF-1 production,
characterized in,
that at least one stimulator of peripheral extrahepatic IGF-1 gene expression is administered to a patient, optionally simultaneously with intermittent administration of GH.

27. Method to specifically stimulate anabolism in peripheral target organs of growth hormone with a concomitant small effect on the circulating levels of IGF-1,
characterized in,
that at least one stimulator or peripheral extrahepatic IGF-1 gene expression is administered to a patient, optionaly simultaneously with intermittent administration of GH.

28. Use of inhibitors of hepatic IGF-I production for the manufacture of pharmaceuticals for reducing fat mass.

29. Use of IGF-I receptor antagonists for the manufacture of pharmaceuticals for reducing fat mass.

30. Use of agonists binding to and activating the same receptors as estradiol for inhibiting liver IGF-I production for the manufacture of pharmaceuticals for reducing fat mass.

31. Use of small nucleic acid molecules for inhibition of liver IGF-I production for the manufacture of pharmaceuticals for reducing fat mass.

32. Use of IGF-I receptor antagonists for the manufacture of pharmaceuticals for reducing retinopathy in diabetic patients.

33. Use of agonists binding to and activating the same receptors as estradiol for inhibiting liver IGF-I production for the manufacture of pharmaceuticals for reducing retinopathy in diabetic patients.

34. Use of small nucleic acid molecules for inhibition of liver IGF-I production for the manufacture of pharmaceuticals for reducing retinopathy in diabetic patients.

5

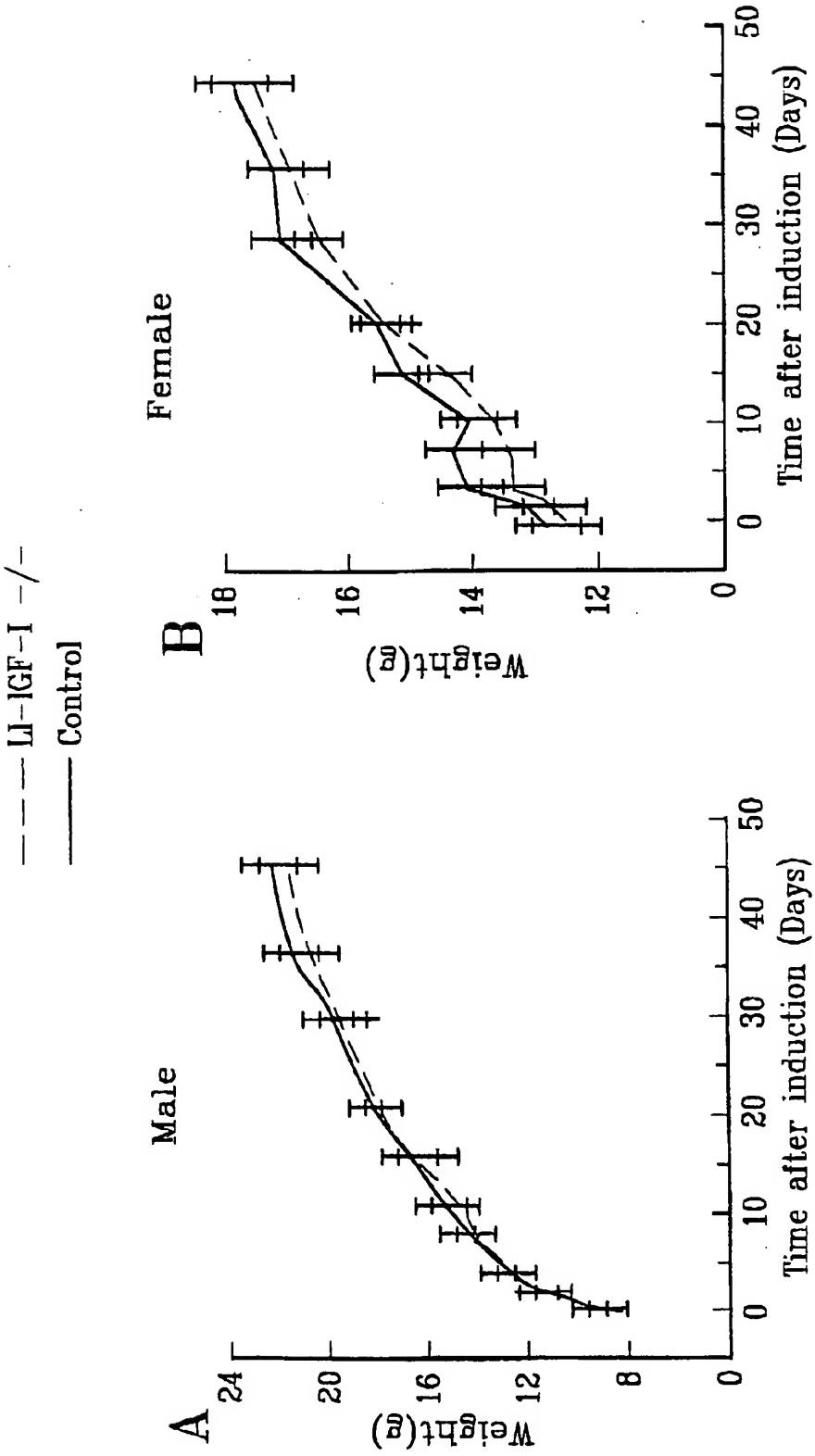
LI-IGF-I -/-
Control
A
Male
Weight(g)
Time after induction (Days)
B
Female
Weight(g)
Time after induction (Days)

FIG. 1

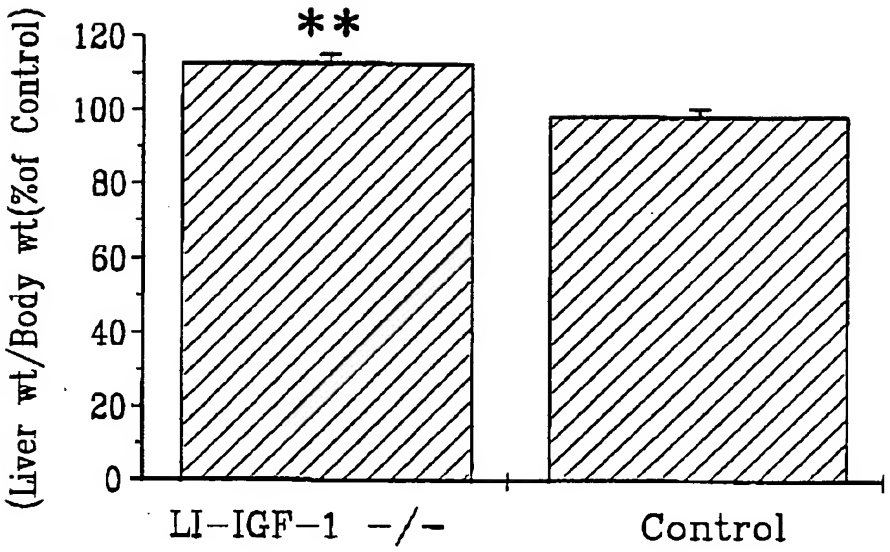
**
120
100
80
60
40
20
0
(Liver wt/Body wt(%of Control)
LI-IGF-1 -/-
Control

FIG.2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/00391

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 38/30, A61K 31/565, A61P 3/04, A61P 35/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9422466 A1 (SYNERGEN, INC.), 13 October 1994 (13.10.94), see the claims --	12-17,19-21, 28,29,30-32, 34
X	US 5473054 A (BRADFORD A. JAMESON ET AL), 5 December 1995 (05.12.95), see column 6 --	12-17
X	US 5840673 A (LEONARD R. BUCKBINDER ET AL), 24 November 1998 (24.11.98) --	12-17,19-21
X	US 5798348 A (MARIA ALEMANY), 25 August 1998 (25.08.98) --	18,30

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 July 2000	13 -07- 2000
Name and mailing address of the ISA/ Swedish Pat nt Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf/EÖ Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/00391

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5661143 A (D'AMATO ET AL), 26 August 1997 (26.08.97), see column 2 -- ---------	18,33

INTERNATIONAL SEARCH REPORT

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/00391

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-11,22-27
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.
PCT/SE 00/00391

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9422466 A1	13/10/94	AU 6626794 A	24/10/94
		CA 2160154 A	13/10/94
		CN 1134111 A	23/10/96
		EP 0708655 A	01/05/96
		FI 954805 A	15/11/95
		JP 8508296 T	03/09/96
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		EP 0639981 A	01/03/95
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		EP 0871475 A	21/10/98
		IL 123383 D	00/00/00
		NO 981127 A	13/03/98
		WO 9709998 A	20/03/97
US 5798348 A	25/08/98	EP 0771817 A	07/05/97
		ES 1031812 U,Y	16/02/96
US 5661143 A	26/08/97	US 5892069 A	06/04/99
		AU 7450994 A	28/02/95
		CA 2168850 A	16/02/95
		EP 0713393 A	29/05/96
		JP 9501433 T	10/02/97
		US 5504074 A	02/04/96
		WO 9504535 A	16/02/95